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Original Article

Vitamin D levels and rickets indices among infants and their nursing mothers in Tripoli – Libya.

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Abstract

This study was conducted to assess the status of vitamin D and rickets indices among healthy infants and their nursing mothers in the city of Tripoli – Libya. A random sample of 110 pairs were studied; 50 infants were exclusively breastfed, 30 were formula fed and 30 received mixed type of feeding. Factors related to vitamin D status such as alkaline phosphatase, parathyroid hormone, calcium, and phosphorous levels were determined. The results showed that, 67 out of the 110 (60.9%) of the infants were vitamin D deficient. The mean level of blood serum vitamin D was 21.3 ± 6.8 nmol/ L. This was accompanied by elevated parathyroid hormone (PTH) (162.5 ± 119.7 pg/ml) P value = 0.001 and alkaline phosphatase (ALP) (394.7±226.1 U/L) p value = 0.001. Calcium and phosphorous remained at normal levels and considered as not dependable indices. The risk increased among exclusively breastfed infants where 40 out of 50 (80%) of them had a mean 25 - hydroxyvitamin D (25(OH)D) of 22.7±8.8 nmol/L while; 10 out of 30 (33.3) of formula fed infants were deficient with a value of 34.7 ± 9.6 nmol/L, and 17 out of 30 (56.7%) who were on mixed type feeding were deficient with an average of 31.0 ± 11.9 nmol/L. Also; 55 out of 80 (69%) of nursing mothers showed hypovitaminosis D with mean blood serum 25(OH)D of 19.8 ± 5.5 nmol/L. This was accompanied with elevated PTH and ALP (P = 0.001), while calcium and phosphorous were not related: P = (0.787) and (0.427). Results of 25(OH)D in nursing mother's milk, showed that 78% of the mother's milk contained less than 140 IU of vitamin D/L of milk. The results indicate that; there is high prevalence of vitamin D deficiency among Libyan infants and their nursing mothers. The risk increases among exclusively breast-fed infants due to low vitamin D levels in nursing mother's milk and low attitude for taking Vit. D supplements.

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Introduction

Vitamin D deficiency is the most common cause of rickets which is still a public health problem in many countries despite the availability of cheap and effective means to prevent it. Vitamin D levels are expressed in the literature by different terms, normally: IU, µg or nmol/ l. The relation between these terms are:1µg = 40 IU and 1ng/ ml = 0.4006 nmol/ l. Vitamin D deficiency was defined as serum levels < 30nmol/ l. (Holick; 2006).

Vitamin D is transported to the liver where it is hydroxylated to 25-hydroxyvitamin (25(OH)D or Calcidiol); the major circulating form of vitamin D. The levels of 25(OH)D in the blood serum reflects total body stores of vitamin D, and it is used to assess vitamin D status (Pettifor; 2004) . In the kidney; 25(OH)D is hydroxylated to the biologically active form of vitamin D, 1,25-dihydroxyvitamin D (1,25-$(OH)_2$ D or (Calcitriol). The actions of 1,25-$(OH)_2$D are to: (i) enhance absorption of calcium and phosphorous from the small intestine; (ii) maintain serum calcium concentration, both directly and through parathyroid hormone; and (iii) promote bone mineralization (Gropper *et al.*; 2005).

Infants with vitamin D-deficiency rickets have a normal serum calcium and phosphorus levels, elevated alkaline phosphatase, and low 25(OH)D levels. Secondary hyperparathyroidism stimulates the kidneys to produce 1,25(OH)D which is the active form of vitamin-D, and thus, 1,25 (OH) levels are normal or often elevated, therefore; the measurement of 1,25 $(OH)_2$D is of no value in determining the state of vitamin D deficiency (Gungor; *et al.*; 2008).

In the early infancy, vitamin D status of breast-fed infants depends mainly on its stores gained across the placenta during intrauterine life (Holick, 2004). Breast-fed infants are more vulnerable, because human milk is a poor source of vitamin D. It contains about 5 - 136 IU/L compared to the adequate intake of 400 IU /day according to the American Academy of Pediatrics (Henderson, 2005). This amount of vitamin D will prevent breastfed infants from developing rickets; a disease thought to be part of the past but unfortunately it is reemerging by new risk factors, such as low maternal 25(OH)D status which is related to low educational level, insufficient intake of vitamin D in the diet, dressing habits and air pollution (Atiq *et al.,* 1998; Marwaha *et al.,* 2011 and Mithal *et al.,* 2009).

Exclusively breastfed infants who do not receive supplemental vitamin D or adequate sunlight exposure are at increased risk of developing vitamin D deficiency (Balasubramanian and Ganesh, 2008; Cabezuelo *et al.,* 2007) because; human milk is a poor source of vitamin D (Dong Shi *et al.*, 2011 and Hollis and Wagner, 2004). Dong Shi *et al.*, (2011) have analyzed the chemical

composition of 66 breast milk samples at three lactation stages in Inner Mongolia, China. The results demonstrated that only 20 of these samples contain vitamin D.

A study in The United Arab Emirates on 33 Emirates women , 25 non Gulf Arab women and 17 European women all residing in the UAE showed that the mean serum 25(OH)D concentrations were in the UAE (21.5 nmol/L) , in non-Gulf Arab women (31.5 nmol/L) and in Europeans residing in the UAE (160 nmol/L). These results were related to the extent of UV skin exposure which depends on the degree of clothing and duration of sunshine exposure (Dawodu; 2004)

Infant daily consumption of breast milk was found to be 780 ml per day. This volume supplied infants with only 0.47 µg of vitamin D which doesn't meet even the 2003 Daily Recommended Intake (DRI_s) of 5µg (200 IU) (Kamao *et al.*; 2007). In November 2008, the American Academy of Pediatrics (AAP) doubled the recommended daily intake of vitamin D for infants and children, from 200 IU/day (2003 recommendation) to 400 IU/day.

However; in a study by Thomson *et al.*; (2004) on 47 mother-infant pairs found 47 mothers had serum 25-(OH)D levels <50 nmol/L, and 39(83%) had levels <30 nmol/L. They also found that most of mothers who were vitamin D-deficient in pregnancy were also deficient postnatally, and their infants, especially if breast fed, were at high risk of vitamin D deficiency. Their study showed that (75%) of breastfed infants were vitamin D deficient compared with (14%) of mixed- fed infants (fed both breast milk and formula milk) and none of formula milk infants were vitamin D deficient. A study conducted by Perrine *et al.*; (2010) aimed to evaluate vitamin D intake among US infants found that most infants are not receiving adequate amount of vitamin D according to the 2008 AAP recommendation. It suggests that Pediatricians and health care providers should encourage parents of infants who are either breastfed or consuming less than one liter per day of infant formula to give their infants an oral vitamin D supplement of at least 400 IU/day.

Libya is a country with abundant sun light, which doesn't encourage pediatricians and obystricians to prescribe vit. D supplements. This doesn't mean the absence of hypovitaminosis D. Because of the absence of any national policy to enrich foods with Vit.D and the recent changes in life style factors such as time spent outdoors, use of sun screen, skin pigmentation and clothing practices as Islamic society (most women wear Hijab) may lead to hypovitaminosis D . Since there are no previous reports on vitamin D levels in Libyan infants or lactating mother's milk; this study was conducted in Tripoli – Libya with the following objectives:

1. To assess vitamin D status among healthy Libyan nursing mothers and their breastfed infants in the city of Tripoli-Libya.

2. To compare vitamin D status between exclusively breast fed, mixed fed (breast and formula- fed) and only formula-fed infants.
3. To assess the subclinical risk factors of vitamin D deficiency.
4. To estimate vitamin D levels in the Libyan nursing mother's milk.

Materials and Methods

The study was designed to involve healthy nursing mothers and their healthy infants at the age of six months. Announcements for the study were placed in most of Primary Health Care centers during the vaccination session of infants at 4 months of age. Those announcements explained the need and importance of this study and its key points. More than 300 volunteers were chosen and scheduled for appointments.

During the six months age vaccination session for infants; a semi-random sample of only 110 infants (55 females and 55 males) and their 110 healthy nursing mothers were selected during the period of 1st of December 2010 till the 10th of January 2011.

Out of the 110 infants; 50 were breastfed, 30 formula-fed and 30 were fed both (formula and breast milk). Data were collected through pre-designed questionnaire that included type of feeding, supplementation, health conditions, anthropometric measurements, and socio-demographic data. Blood samples were collected from all infants and their mothers (lactating only) for the determination of 25(OH)D, parathyroid hormone, alkaline phosphatase, calcium, phosphorous and (Hemoglobin) to ensure healthy nutritional status). Milk samples were collected from lactating mothers for 25(OH)D analysis (see Fig. 1).

All blood tests were done at Saint James Hospital, Tripoli - Libya; Using An electrochemiluminescence Immunoassay (ECLIA) Kit and Immunoassay analyzer Cobas - e411 (Roche- Germany) for 25(OH) D and, parathyroid hormone and Using I lab 600 chemistry System Kit from Instrumentation Laboratory- Italy, for alkaline phosphatase and calcium while phosphorous was determined by using I lab 300 plus chemistry system from Instrumentation Laboratory- Italy.

Blood Hemoglobin was determined using COULTER® A^c●T diff 2™ Analyzer

Vit. D levels in milk were determined in the faculty of Agriculture and Nutrition in the United Arab Emirates University; Alain UAE by UHPLC according to a modified method of Escriva *et al.*; (2002) and Korchazhkina *et al.*; (2006). Data were entered using Epi Info 3.5.3 and analyzed by using SPSS software.

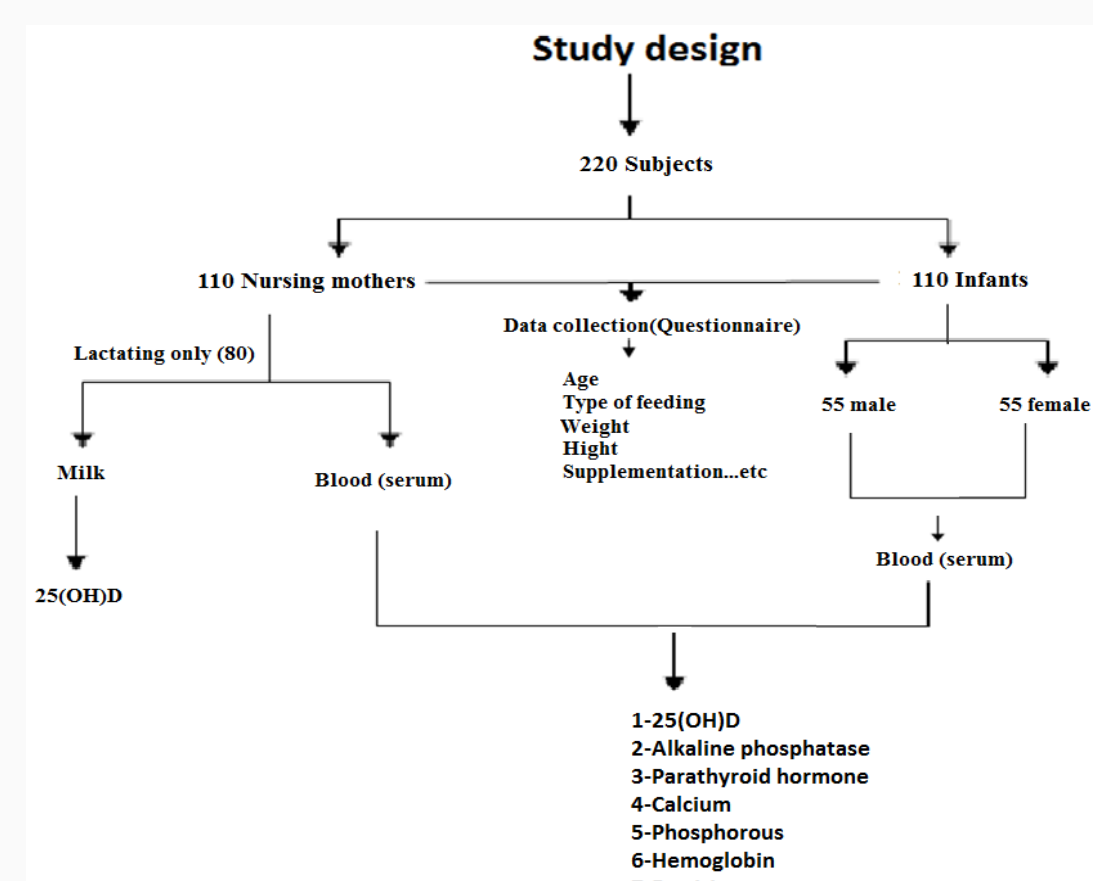

Fig.1 Study design

Results and Discussion

Table 1. shows some of the anthropometries for the infants and their nursing mothers, and the levels of 25(OH)D and Hemoglobin. Infant's height (length) and weight are considered normal according to WHO charts. Also; hemoglobin level for both the infants and their mothers was within normal range which indicates a good general health and nutritional status. But serum 25(OH)D levels were low in both infants and their nursing mothers.

Table 2. Shows that, 60.9% of the total number of infants were deficient in vitamin D (≤ 30nmol/L) with mean ±SD (21.3 ± 6.8 nmol/L) while; Thomson et al. (2004) reported that 75% of breastfed infants were vitamin D deficient compared with (14%) of mixed- fed infants (fed both breast milk and formula milk) and none of formula milk infants were vitamin D deficient.

Table 1. Sample description.

Parameter	Mothers (mean ± SD)	Infants (mean ± SD)
Number	110	110
Age	30.2 ± 5 years	≈ 6 months (192.2 ±13.2 days)
Birth weight (Kg)	-	3.31 ± 0.5
Current weight (Kg)	76.6 ± 12.2	8.03 ± 0.8
Height (cm)	162.3 ± 4.2	70 ± 4.1
25 (OH)D nmol/L	24.7±8.8 (80 mothers)	28.2 ± 11.2
Hemoglobin (g/dl)	13.1±0.95 (80 mothers)	12.05 ± 0.83

Normal ranges for Hemoglobin, Infant: 9-14 g/d ; Adult female: 12 – 16 g/dl.

Table. 2. Blood Serum 25(OH)D levels for the total number of infants.

parameter	Number (%)	25(OH)D(nmol/L) Mean ±SD	Range
Deficient *	67 (60.9%)	21. 3 ± 6.8	7 – 30
Non-deficient **	43 (39.1%)	38.2 ±8.3	30.1-59

*** Deficient ≤ 30 nmol/L, ** Non deficient > 30**

Table -3- summarizes the biochemical indicators for vitamin D deficient and non- deficient infants. Results indicate the presence of an inverse relationship between 25(OH) D levels and the levels of alkaline phosphatase and parathyroid hormone (P<0.0001, r = - 0.0556 and P<0.0001, r = - 0,642 respectively); but there is no relation between 25(OH) D and calcium or phosphorous levels (p = 0.787, r = 0.27 and P = 0.427, r = - 0.076 respectively).

Table 4. shows high prevalence of hypovitaminosis D among exclusively breastfed infants (80%) compared with formula fed (33.3%) and (56.7%) for infants fed both (P =0.001).

Table 3. The relation between total infants 25(OH) D status and their blood serum biochemical indices.

Parameter	Infants 25(OH)D status	Number of infants	Mean ±SD	Range	P and r values
Alkaline phosphatase(U/L)	Deficient	67	394.7 ± 226.1	111 - 1437	P < 0.0001
	Non deficient	43	254.3 ± 72.4		r = - 0. 556
Calcium (mg/dl)	Deficient	67	9.8 ± 0.63	7.7 - 11.8	P = 0,787
	Non deficient	43	9.7 ± 0.71		r = 0.270
Phosphorous (mg/dl)	Deficient	67	5.8 ± 1.2	2.9 - 8.8	P = 0.427
	Non deficient	43	4.4 ± 0.99		r = - 0.076
Parathyroid hormone (pg/ml)	Deficient	67	162.5 ± 119.7	13.1 - 652.4	P < 0.0001
	Non deficient	43	56.0 ± 26.1		r = - 0. 64

Values with the same superscript in the same raw are not statistically different at 0.05% level.

Normal ranges: ALP- 145-420 U/l., Ca- 8.5-10.5 mg/dl., Phos- 2.5-4.5 mg/dl., PTH- 10-60 pg/ml.

Table 4. Effect of type of feeding on infant's serum vitamin D status.

Type of feeding	Infant vitamin D status (numbers of total)		Total
	Deficient (≤ 30 nmol/L)	Non deficient (> 30 nmol/L)	
Breast feeding	40 (80%)	10 (20.0%)	50 (100.0%)
Formula-feeding	10 (33.3%)	20 (66.7%)	30 (100.0%)
Feeding –both	17 (56.7%)	13 (43.3%)	30 (100.0%)
Total	67 (60.9%)	43 (39.1%)	110 (100.0%)

Table.5. Shows significant differences in infant's serum 25(OH)D and its related biochemical factors as affected by their feeding patterns. Formula fed infants had highest mean of 25(OH)D levels, followed by infants fed both (formula and breast milk), while breast fed infants had the lowest level

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of 25(OH)D. On the other hand breast fed infants had highest levels of alkaline phosphatase and parathyroid hormone, compared to formula fed and infants who were fed both, while no significant differences observed in calcium and phosphorous levels. In agreement with these results, a high prevalence of vitamin D deficiency has been reported among breast-fed infants. Seventy five percent of the breast fed infants in Iowa - USA and 80% of the neonates in Ankara - Turkey were vitamin D deficient. (Abrams; 2011 and, Ziegler; 2006). In this study 60.9%, of the breastfed infants were vitamin D deficient.

Table 5. Effects of type of feeding on infants vitamin D status and its related biochemical factors.

Blood Tests	Breast- fed infants		Formula fed infants		Infants fed- both	
	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range
25(OH)D (nmol/L)	22.7 ± 8.8 [a]	7 – 39	34.7 ± 9.6 [b]	20-59	31.0± 11.9 [b]	9 - 58
Calcium (mg/dl)	9.8± 0.7 [a]	7.7 - 11.8	9.6 ± 0.65 [a]	8.5-10.7	9.8 ± 0.6 [a]	8.4 - 11.6
Phosphorus (mg/dl)	5.6 ± 1.3 [a]	2.9 - 8.8	5.7± 10 [a]	3.6-7.3	5.5 ± 0.8 [a]	3.9 - 7.1
ALP* (U/L)	432 ± 248.9 [a]	158 – 1437	243 ± 59.4 [b]	111-329	282 ± 75 [b]	163 – 513
PTH** (pg/ml)	170 ± 125 [a]	25.6 - 652.4	66 ± 31.6 [b]	19.6 -139.7	94.0 ± 90 [b]	13.1 – 457

*ALP- Alkaline Phosphatase enzyme.

**PTH- Parathyroid hormone.

a,b: means with the same letter in the same row are not statistically different (P<0.05).

Also; the results indicate that, 69% of the mothers are suffering from vit.D deficiency with a blood serum 25(OH) D level of 19.8± 5.5 nmol/L (Table 6); while Thomson *et al.*; (2004) found the level in 83% of nursing mother's milk <30 nmol/l. This agrees with other studies in Ankara (Turkey), Karachi (Pakistan) and Zanjan (Iran) where 46%, 45% and 66% of the studied women were vitamin D deficient (Atiq *et al.;* 1998; Kazemi *et al.*, 2009 and Andiran *et al.*, 2002). A number of factors are probably responsible for these observations, such as; lack of foods fortification with vit.D in Libya, the preference of the women to avoid direct sunlight to avoid skin discoloration, religion and social obligations for the women to cover their skin when going outside and the lack of taking vit.D supplements.

Table 6. Lactating mothers blood serum 25(OH)D status.

Parameter	Number	25(OH)D (nmol/L)	
		Mean±SD	Range
Deficient 25(OH)D≤ 30 nmol/L	55 (69 %)	19.8 ± 5.5	9 – 30
Non deficient 25(OH)D >30 nmol/L	25 (30 %)	35.4±3.8	31 – 45

However; no relation was found in vitamin D status between mothers and their nursing infants at six months of age. Among none vitamin D deficient mothers, 80% of their nursing infants were vitamin D deficient; while among deficient mothers only 67.3% of their nursing infants were vitamin D deficient (P = 0.295).

Elevated parathyroid hormone and alkaline phosphatase were also common among mothers, and an inverse relationship between 25(OH)D and PTH was observed (Table 7); which is in agreement with the findings of Marawaha et al; (2011). The relation of mother's 25(OH) D status to blood serum alkaline phosphates was P= 0.001 and r = - 0.371; to calcium was P= 0.767 and r = - 0.034; to phosphors was P= 0,037 and r = - 0.233 and to parathyroid hormone was P=0.001 and r = - 0.441. These results are in agreement with the results of the relationship between infants 25(OH) D and biochemical indices (table 3).

Gropper *et al.*; (2005) reported that only 10% of diet calcium can be absorbed in the absence of vitamin D; thus parathyroid levels will be elevated to promote the withdraw of calcium and phosphorous from bones which may lead to developing osteoporosis (Henderson, 2005 and Mithal et al.; 2009).

Table 7. The relation between total mothers 25(OH) D status and their blood serum biochemical indices.

Parameter	25(OH)D status	Number	Mean ± SD.	Range
Alkaline phosphatase (U/L)	Deficient	55	137.8 ± 54.96	58 – 297
	Non deficient	25	101.2 ± 27.80	58 – 152
Calcium (mg/dl)	Deficient	55	8.60 ± 0.63	7.1 – 10
	Non deficient	25	8,64 ± 0.69	7.1 – 10
Phosphorous (mg/dl)	Deficient	55	3.4 ± 0.59	2.1 - 4.
	Non deficient	25	3.3 ± 0.65	3.2 - 4.4
Parathyroid hormone (pg/ml)	Deficient	55	126 ± 43.5	39.9 – 210
	Non deficient	25	83.2 ± 31.4	36.3 – 166

Normal ranges: ALP- 30-100 U/L; Ca - 8.5-10.5 mg/dl; Phos- 2.5-4.5 mg/dl; PTH- 10-60 pg/ml.

Hence; a large proportion of the infants and nursing mothers were vitamin D deficient, having blood serum vitamin D below 30 nmol/L, whereas the sufficient level considered to be ≥ 50 nmol/L. (Marwaha; *et al.*; 2011 and Misra *et al.*; 2008). Exclusively breastfed infants were the most vulnerable group for vitamin D deficiency.

Since breast milk is a poor source of vitamin D (Andiran *et al.*; 2002 and Dong Shi *et al.*; 2011), thus the American academy of pediatrics (APP) recommends the use of vitamin D supplements of 400 IU/day starting in the first few days of life for all breast-fed infants and infants receiving less than 500 ml/ day of fortified formula milk (Henderson; 2005 and Kazemi *et al.*; 2009). Accordingly; breastfed infants who do not receive any vitamin D supplementation are under a very high risk of hypovitaminosis D which may lead to the development of rickets and other related diseases to vitamin D deficiency such as cancer, cardiovascular disease, type 1 diabetes mellitus, multiple sclerosis, depression and schizophrenia (Holick; 2004 and Norman; 2008).

From Fig.2; the level of 25(OH) D in 77.6% of mother's milk was 140 IU/L or less and was not detected in 21.3% of the samples. This agrees with the results of Dong Shi *et al.*; (2011) in Inner Mongolia, China; where only 20 out of 66 breast milk samples contained vitamin D. This indicates that 77.6% of the nursing mothers will not provide their nursing infants with their requirements of vitamin D. Also; in this study a significant relationship between type of residence and vitamin

D status was observed (data not shown) as women who live in a farm or a villa (house with a garden) had better vitamin D status which may be due to a better opportunity to enjoy daily outdoor activity.

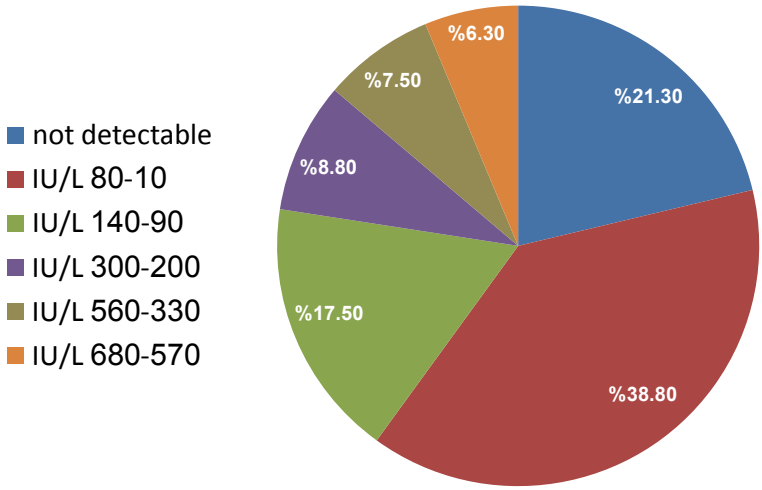

Fig. 2. 25(OH)D3 level in milk of lactating mothers (IU/L)

Conclusion

Vitamin D supplementation should be recommended to all breast-fed, mixed formula and breast-fed infants and nursing mothers. It is necessary to evaluate vitamin D status among Libyans. Evaluation of vitamin D status for infants and nursing mothers in other parts of Libya plus long term strategies to prevent hypovitaminosis D should be implemented. Measures could include; vit.D supplementation, public education, fortification of foods with vitamin D and modification of food habits.

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مستوى فيتامين "د" ومؤشرات مرض الكساح عند الأطفال الرضع وأمهاتهم بمدينة طرابلس – ليبيا.

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2 - قسم الأطفال - مستشفى الجلاء - طرابلس - ليبيا.

المستخلص

أجريت هذه الدراسة لتقدير مستوى فيتامين "د" بين الأطفال الرضع الأصحاء الليبيين وأمهاتهم المرضعات في مدينة طرابلس لعدد 110 رضيع وأمهاتهم ، كما تم دراسة العوامل المرتبطة بوضع فيتامين "د" مثل الألكالين فوسفاتيز، هرمون الغدة جار الدرقية، الكالسيوم والفوسفور. أظهرت النتائج أن 60.9% من الأطفال كان لديهم نقص في مستوى فيتامين "د"؛ حيث وجد أن معدل فيتامين "د" في مصل الدم (21.3± 6.8 نانومول/لتر). هذه النتائج كانت مترافقة مع ارتفاع في مستوى هرمون الغدة جار الدرقية (162.5 ± 119.7 بيكوجرام/ مللي لتر) وبمستوى احتمالية 0.001 وكذلك ارتفاع في مستوى الألكالاين فوسفاتيز(394.7±226.1 وحدة / لتر) وبمستوى احتمالية 0.001، في حين بقيت كل من مستويات الكالسيوم والفوسفور ضمن المعدلات الطبيعية واعتبرت مؤشرات سلبية. ازداد خطر نقص فيتامين "د" بين 40 من أصل 50 من الأطفال المعتمدين على الرضاعة الطبيعية فقط ؛ أي: بنسبة (80%) منهم؛ حيث كان مستوى فيتامن "د" 22.7±8.8 نانومول/لتر. أما الأطفال المعتمدين على الرضاعة الصناعية فكان نقص فيتامين "د" بين 10 من أصل 30 طفلا (33.3%) وكان مستواه 34.7±9.6 نانومول / لتر؛ بينما بين الأطفال المعتمدين على الرضاعة المختلطة كان النقص بين 17 من أصل 30 طفلا (56.7%) وكان مستواه 31.0 ± 11.9 نانومول/لتر. أظهرت النتائج- أيضا- أن 55 من أصل 80 (69%) من الأمهات المرضعات كان لديهن نقص في مستوى فيتامين "د" في الدم بمعدل 19.8 ±5.5 نانومول/لتر، وهذا – أيضا- كان مصحوبا بارتفاع في مستوى هرمون الغدة جار الدرقية والألكالين فوسفاتيز بمستوى احتمالية 0.001. بينما بقى مستوى الكالسيوم والفوسفور ضمن المعدلات الطبيعية بمستوي احتمالية (0.787) و (0.427) لكل منهما. أظهرت نتائج تحليل فيتامين "د" في حليب الأمهات المرضعات أن 77.6% من الأمهات يحتوي حليبهن على 140 وحدة دولية / لتر من فيتامين " د" أو اقل. أشارت نتائج هذه الدراسة إلى أن نقص فيتامين " د" منتشر بشكل كبير بين الأطفال الليبيين وأمهاتهم المرضعات بمدينة طرابلس ، ويزداد الخطر بين الأطفال المعتمدين على الرضاعة الطبيعية بسبب نقص مستوى فيتامين "د" في حليب الأمهات المرضعات وعدم الرغبة في استعمال مكمل فيتامين "د".

الكلمات الدالة: فيتامين "د"، الكساح، الأطفال الرضع، الأمهات المرضعات.

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